FORMALDEHYDE TRACES AND QUALITY CHARACTERISTICS OF INDIAN MACKEREL (*Rastrelliger kanagurta)* FROM MARKETS IN KOTA BHARU

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CERTIFICATION

This is to certify that we have read this research paper entitled '**Formaldehyde Traces and Quality Characteristics of Indian Mackerel (*Rastrelliger kanagurta*) from Markets in Kota Bharu, Kelantan**' by Hari HaraPrien K. Kesavan, and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course DVT 5436 – Research Project.

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List of Symbols and Abbreviations

cm – Centimetres

g - Grams

kg - Kilogrammes

km - Kilometres

kV – Kilovolts

m - Metres

mm – Millimetres

ml – Millilitres

N – Normality

µL - Microlitres

µm - Micrometres

% - Percentage

° C – Degree Celsius

GC-MS – Gas Chromatography Mass Spectrometry

HCl – Hydrochloric Acid

KOH – Potassium Hydroxide

ppm – Parts Per Million

QIM – Quality Index Method

TCA – Trichloroacetic Acid

TMAO – Trimethylamine Oxide

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ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 5436 – Research Project

Formaldehyde is a naturally occurring organic compound found to be present in fish, yet higher traces formaldehyde has significant effect on health of the consumers and quality characteristics. This study is conducted to analyse the formaldehyde traces and quality characteristics in Indian Mackerel (*Rastrelliger kanagurta)* in Malaysia. A total of six Indian Mackerel samples was randomly collected from Siti Khadijah Wet Market and AEON Mall Supermarket located in Kota Bharu, Kelantan. Formaldehyde was extracted from the fish samples using Trichloroacetic acid followed by the Gas Chromatography – Mass Spectrometry (GC-MS) method for qualitative analysis of formaldehyde traces. There were no traces of formaldehyde present in all six Indian Mackerel samples. The quality characteristics of the Indian Mackerel was assessed using the sensory quality assessment and pH to determine the differences in fish qualities obtained from Siti Khadijah Wet Market and AEON Mall Supermarket. Sensory quality was assessed based on characteristics such as odour, appearance and texture of the Indian Mackerel along with the pH. The result analysis has shown that Siti Khadijah Wet Market has higher degree of fish quality compared to AEON Mall Supermarket. The amount of formaldehyde obtained was compared with at most 5 ppm which is regulated by The Food Act 1983 and The Food Regulations 1985 of Malaysia. Surprisingly, this study recorded no formaldehyde in purchased Indian Mackerel in either wet market or supermarket indicating it is safe for human consumption.

Keywords: *Indian Mackerel, Formaldehyde, Quality Characteristics, GC-MS, Human Consumption, Kota Bharu*

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 5436 – Projek Penyelidikan.

Formaldehid adalah sebatian organik semula jadi yang terdapat pada ikan, namun tahap formaldehid yang lebih tinggi mempunyai kesan yang signifikan terhadap kesihatan orang awam dan ciri kualiti. Kajian ini dilakukan untuk menganalisis tahap formaldehid dan ciri kualiti dalam Ikan Kembung (*Rastrelliger kanagurta*) di Malaysia. Sebanyak enam sampel Kembung diperolehi secara rawak dari Pasar Basah Siti Khadijah dan Pasaraya AEON Mall yang terletak di Kota Bharu, Kelantan. Formaldehid diekstrak dari sampel ikan menggunakan asid trikloroasetik diikuti dengan kaedah Gas Chromatography-Mass Spectrometry untuk analisis kualitatif tahap formaldehid. Keputusannya, tiada formaldehid yang terdapat dari keenam-enam sampel Kembung. Ciri-ciri kualiti Kembung dinilai menggunakan penilaian kualiti dan pH untuk menentukan perbezaan kualiti ikan yang diperoleh dari Pasar Siti Khadijah dan Pasaraya AEON Mall. Kualiti deria dinilai berdasarkan ciri-ciri seperti bau, penampilan dan tekstur Kembung bersama dengan pH. Hasil analisis menunjukkan bahawa Pasar Siti Khadijah mempunyai tahap kualiti ikan yang lebih tinggi berbanding dengan Pasaraya AEON Mall. Jumlah formaldehid yang diperoleh dibandingkan dengan paling banyak 5 ppm yang dinyatakan dalam Akta Makanan 1983 dan Peraturan – Peraturan Makanan 1985 Malaysia. Anehnya, kajian ini tidak mencatatkan formaldehid dalam ikan kembung yang dibeli di pasar basah atau pasaraya. Maka, ia selamat untuk dimakan oleh manusia.

Kata Kunci: *Ikan Kembung, Formaldehid, Ciri-ciri Kualiti, GC-MS, Pemakanan Manusia, Kota Bharu*

1.0 INTRODUCTION

The Malaysian population is familiar with the fish species known as "Ikan Kembung," also known as Indian Mackerel. Indian Mackerel belongs to the Scombridae family and Rastrelliger genus. It is also known as *Rastrelliger kanagurta* scientifically. The Indo-West Pacific is home to a large population of this fish species (Luther, 1995; Amin *et al.*, 2014). As reported by Noble *et al.*, (1992), the abundance of schools of *Rastrelliger kanagurta*, notably in Asian waters, is also the foundation of significant commercial fishes for human consumption. There are numerous reasons for this. To begin with, Malaysians like Indian Mackerel, which is locally commercialised and available in a variety of forms, including fresh, salted, smoked, and dried (Rumpet *et al.*, 1997; Ambak *et al.*,2010; Amin *et al.*, 2014). Additionally, Malaysians consume a lot of Indian Mackerel because of its incredibly low-price range, accessibility, and abundant availability in marine fish catches (Rahman & Hafzath, 2012).

The fish is a highly demanded commodity in Malaysia. According to, Star Asia News Network (2014), Malaysians, typically consume at least 56.5 kilograms of fish per person annually, are among the world's greatest consumers of fish The three most eaten fish are catfish, tilapia, and mackerel. And as Malaysia's population expands, the rate of consumption is anticipated to rise proportionally. The fish is rich in fat, free amino acids and water making it highly susceptible to spoilage due to microbial proliferation and biochemical reactions during post-mortem process (Fernandes & Venkatraman, 1993; Ismail, 2005). Hence, fish and seafood depending on the species, have a higher tendency to spoil and can be kept fresh in ice for 8 to 14 days.

The overall catch of marine fish has a significant impact on the fish market. In accordance with the Department of Fisheries Malaysia (2019), the combined inshore and deep-sea catch of Kelantan is only 90,205 Tonnes. But out of all the states in Malaysia, Perak has the highest number of catches, with 314,957 Tonnes. Due to the less catches, it is believed that the fisherman in Kelantan tend to preserve their fish catches fresh for as long as the fish carcasses could last to be sold for profit and to satisfy the customer demand with the use of preservatives. Thus, fishermen and fish merchants could be using formaldehyde recklessly as a preservative agent to maintain the freshness of fish and shellfish. However, due to formaldehyde's carcinogenic nature, high levels of formaldehyde intake can result in respiratory tract disorders and, in the worst situation, lead to the development of cancer in people (American Cancer Society, 2014). Myeloid leukaemia and nasopharyngeal carcinoma are two malignancies frequently linked to formaldehyde exposure (American Cancer Society, 2014).

Formaldehyde (HCHO), also known as methanal, is an organic compound and the most basic of the aldehydes. It is created primarily by the vapor-phase oxidation of methanol and is frequently supplied as formalin, an aqueous solution at a 37 percent concentration (National Centre for Biotechnology Information, 2022). Formaldehyde is considered as a hazardous harsh chemical to the environment and humans. Prioritize proper handling of formaldehyde by trained professionals in laboratories and other sectors.

This chemical is cruel, yet it is also a naturally occurring substance in perishable foods. The levels of naturally occurring formaldehyde in fruit, vegetables, milk, chicken, mutton,

and meat samples were up to 58.3, 40.6, 5.2, 8.2, 15.2, and 8.5 ppm, respectively (Nowshad *et al*, 2018). Cod and crustaceans have naturally occurring formaldehyde levels of 4.6 to 34 mg/kg and 1-98 mg/kg, respectively, in these kinds of seafood (World Health Organization, 2017). There are no data available for every food because there have only been a few thorough research conducted on the quantities of naturally occurring formaldehyde in food. The available research evidence at hand indicates that various fruits and marine fish have the most significant levels of naturally occurring formaldehyde up to 60 mg/kg (Centre for Food Safety, 2017). Additionally, formaldehyde levels in fish are naturally between 6.5-293 mg/kg, while human consumption's acceptable levels are only 100 mg/kg (European Food Safety Authority, 2014).

1.1 Research problem

The trace of formaldehyde is naturally occurring in fish. However, fish contaminated with high levels of formaldehyde can be harmful for human consumption. American Cancer Society has classified formaldehyde as a known carcinogen which could lead to development of various types of cancers. Limited research has been done on formaldehyde trace present on fish in Kota Bharu.

Furthermore, the presence of formaldehyde in the fish is due to enzymatic breakdown of Trimethylamine oxide (TMAO). It eventually affects the carcass of the fish that has not been kept in proper storage leading to degradation of the fish freshness and quality (Bhowmik et al., 2017). Evaluation of the quality characteristics of the fish distinguishes the degree of fish quality.

Finally, till to this day, there is no similar research has been done on Indian Mackerel in Kota Bahru, Kelantan. There are more opportunities to be exploited to study further on

Indian Mackerel to gather more information for documentation purposes. This research creates awareness on the safety of fish for human consumption.

1.2 Research questions

1.2.1 Do the Indian Mackerel acquired from markets have formaldehyde traces?

1.2.2 Are there any differences in Indian Mackerel qualities between the supermarket and the wet market?

1.2.3 Are the Indian Mackerel acquired from markets is safe for human consumption?

1.3 Research hypothesis

1.3.1 There are presence formaldehyde traces in Indian Mackerel obtained from the markets.

1.3.2 The Indian Mackerel obtained from the wet market has a good quality compared to the supermarket.

1.3.3 The Indian Mackerel acquired from markets are safe for human consumption.

1.4 Objectives

1.4.1 To analyse the formaldehyde traces in Indian Mackerel obtained from the markets.

1.4.2 To determine the differences in quality of the Indian Mackerel between the wet market and supermarket.

1.4.3 To determine the Indian Mackerel acquired from the markets are safe for human consumption.

2.0 LITERATURE REVIEW

2.1 Formaldehyde Presence and Development in Fish

Formaldehyde is a naturally occurring organic compound present in various seafood. According to Bhowmik (2017), the level of formaldehyde in the marketed finfish species of various fish markets was found to be in a natural level, which is below the tolerable levels for humans and this study was conducted in Dhaka, Bangladesh where the fish were sampled from selected markets such as Kawran bazaar, Raiyer bazaar, and Mohakhali bazaar. This shows that the natural formaldehyde presence at the fish market is low and safe for consumption for consumers. According to this study, the fish from various markets had a formaldehyde content that ranges from 0.73 mg/kg to 5.1 mg/kg to 7.87 mg/kg to 39.68 mg/kg, which is lower than the baseline to be considered as hazardous to people. However, the storage and handling techniques have an impact on the formaldehyde levels in the fish. It is believed that when fish is transported improperly in large quantities over long distances by the dealers or wholesalers, they often fail to use appropriate amount of ice to maintain the fish's freshness, which leads to the microbial growth and chemical reactions that shorten the shelf life. Consequently, there has been a major decline in fish quality by the time a customer purchases.

After death and during frozen storage, the fish carcass experiences several physiological and chemical changes, particularly quality degradation, which may be seen by examining the texture, flavour, colour, and odour. In agreement with Sotelo *et al.*, (1995) and Bhowmik *et al.*, (2017), the enzymatic reduction of Trimethylamine oxide (TMAO) produces formaldehyde as well as dimethylamine. Theoretically, it is known that protein denaturation in fish is caused by formaldehyde-mediated denaturation. The physical manifestation of this

reaction can be seen when inspecting the texture. This is due to formaldehyde build-up in fish muscle fibre during frozen storage causing extensive denaturation overtime. Thus, it is hypothesised that the primary cause of protein denaturation in the study of gadoid fish was resulted from the generation of formaldehyde during the freezing storage. However, there is no conclusive evidence to support the idea that prove the covalent bonding between proteins and formaldehyde to from crosslinks during the frozen storage. As a result of the post-mortem enzymatic breakdown of trimethylamine oxide (TMAO) in the muscle fibres of frozen saltwater fish contains traces of formaldehyde.

2.2 Formaldehyde Trace Presence in Exported Fish from Asia

The seafood imported from some of the countries proven to be detected for higher levels of formaldehyde presence. According to Andrew (2013), tests conducted by researchers at a North Carolina chemical engineering firm and North Carolina State University revealed that many fish imported from China and Vietnam and sold in at least some United States supermarkets contained unnatural levels of formaldehyde, a known carcinogen. It is believed that Asia is a region where formaldehyde utilization in food is widespread. Additionally, it has been established that the majority of imported fish from Asia contain traces of formaldehyde. Meanwhile, neither the fish from the United States nor any other region has any traces of formaldehyde. The largest exporters of seafood worldwide are China and Vietnam. Long-distance transport of seafood is purposefully infused with formaldehyde to prevent rotting to avoid financial losses and eventually putting the health of the consumer at jeopardy.

2.3 Regulations of Formaldehyde Levels in Fish

Tolerable amounts of formaldehyde for humans are 100 mg/kg, but natural levels of the substance in fish range from 6.5-293 mg/kg (European Food Safety Authority, 2014). The use of formaldehyde is tightly regulated worldwide. Use of formaldehyde has been approved by the U.S. Food and Drug Administration (FDA) to be used as a preservative in defoaming products that contain dimethylpolysiloxane, not exceeding 1.0 percent of the dimethylpolysiloxane content (Code of Federal Regulations Title 21, 2022). The Food Act 1983 and The Food Regulations 1985 of Malaysia, state that formaldehyde cannot be used in seafood products with the exception for smoked fish only, which is allowed only up to a maximum of 5 ppm. Formaldehyde is a chemical that is harmful to human health and is thought to be a human carcinogen. As a result, it is prohibited in consumable food. The use of formaldehyde is illegal in Thailand (Notification No. 151 (B. E. 2536) of the Thai Ministry of Public Health, 28 December 1993) and is listed as a toxin in Singapore and Malaysia. In addition, the Environmental Protection Agency (EPA) of the United States determined that accidental consumption up to 0.2 mg per kg of body weight is still safe.

2.4 Classification of Formaldehyde as Carcinogen

Formaldehyde is classified as a known carcinogen by many reputable health organizations. As stated in Public Health Statement (2008), high amounts of formaldehyde exposure induce a burning feeling mostly in eyes, nose, and lungs. Through consumption of poisoned food and water, formaldehyde enters the body and is quickly absorbed in the gastrointestinal tract. Formaldehyde is quickly converted into various absorbable molecules

within the body. In a study, rats given high oral doses of formaldehyde showed signs of stomach injury (Public Health Statement, 2008). Studies on people who were repeatedly exposed to formaldehyde in the air at work showed higher incidences of nose and throat cancer (Public Health Statement, 2008). In another study, some laboratory rats that were exposed to formaldehyde for their entire lives had developed nose cancer (Public Health Statement, 2008). Based on studies of inhalation exposure in humans and laboratory animals, the Department of Health and Human Services (DHHS) and the International Agency for Research on Cancer (IARC) have classified formaldehyde as a human carcinogen (Public Health Statement, 2008).

3.0 MATERIALS AND METHODS

3.1 Sample sites

The prominent wet market located in Kota Bahru was selected as a sampling site. The study was conducted in Siti Khadijah Market in Kota Bharu with the coordinates of (6.130205495342268, 102.23926454618757) about 8.1 km away from the Faculty of Veterinary Medicine, University Malaysia Kelantan (UMK). For the supermarket, the AEON Mall in Kota Bharu was selected which had the coordinates of (6.111512615391595, 102.23017805851381) about 10.9 km away from the Faculty of Veterinary Medicine, University Malaysia Kelantan (UMK).

3.2 Collection of fish

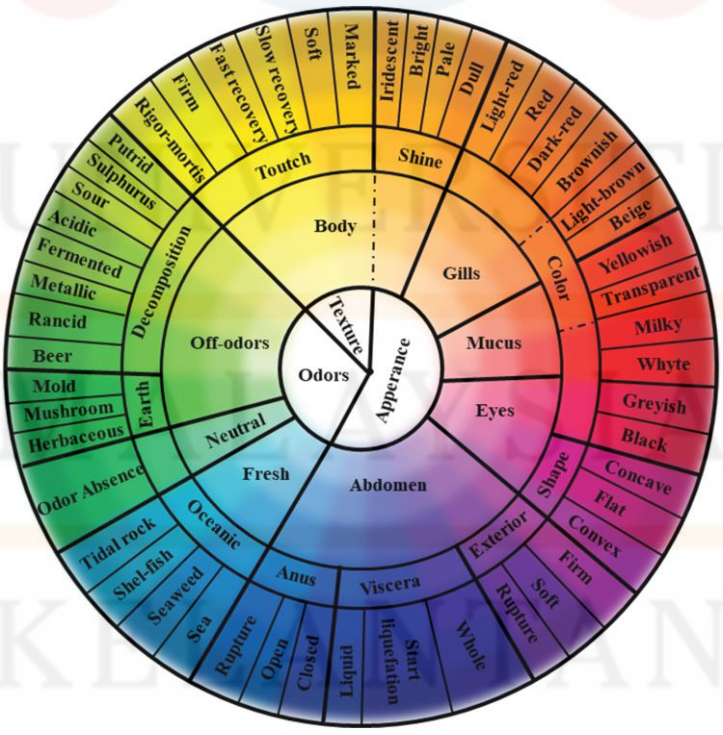
A widely consumed commercial fish species which is Indian Mackerel or scientifically known as *Rastrelliger kanagurta* was selected as target sample. Indian Mackerel is one of the cheapest marine fish in markets. Thus, it has been highly preferred by the locals in Kota Bharu for daily consumption. Each of fish sample weighing 100 g to 350 g was randomly purchased from AEON Mall Supermarket and Siti Khadijah Market respectively in Kota Bharu for three days, respectively. The collected samples were stored in sterile bags and kept in an ice box and brought to the Aquatic Laboratory, University Malaysia Kelantan (UMK). Upon arrival at the laboratory, the length and weight of the fish were measured. The fishes acquired from AEON Mall ranged from 26.8 cm to 28.1 cm in length and 0.25 kg to 0.29 kg in body weight. Meanwhile the fishes acquired from Siti Khadijah Market ranged from 22.3 cm to 28.0 cm in length and 0.13 kg to 0.31 kg in body weight. Then the flesh was separated from the skin and bones without damaging the gut by using a sterile scalpel size 15 for further analysis. Followed

by that, the fishes were assessed for the sensory and meat qualities and spoilage indicators including formaldehyde content was analysed.

3.2.1 Sensory quality assessment

Sensory methods are used to assess the degree of freshness based on the Figure 3.1 Quality Index Method (QIM) sensory wheel (Freitas *et al.*, 2021). The fish was assessed according to three main criteria such as texture, appearance, and odours. For the texture, the body of the fish was assessed. For the appearance, the shine, the colour of gills and the colour and shape of eyes and the abdomen was assessed. Meanwhile the odour of the fish naturally portrays the degree of freshness in the acquired fish samples.

Figure 3.1 QIM sensory wheel.



3.2.2 Determination of pH

A 10 g of fish flesh is weighed. It was homogenized thoroughly with 100 ml distilled water for 5 minutes (Noordiana *et al.*, 2011). The pH of supernatant is measured by means of a Universal Indicator Paper pH 1 – pH 14. The readings were recorded.

3.2.3 Determination of Formaldehyde

The fish samples are thawed at room temperature range between 25 to 28 degree Celsius. The flesh was cut into small pieces and 30 g samples was extracted. Then the sample was homogenised with 60 ml of 6% w/w Trichloroacetic acid (TCA) for 10 minutes. The extracted solution was filtered by a Whatman No.1 filter paper, and the supernatant was collected. The filtrate then adjusted between pH 6.00-7.00 using Potassium hydroxide (0.1N) and hydrochloric acid (0.1N). About five milliliters of the solution was then transferred into a transport container and the sample was kept in a freezer at -20 degree Celsius until further analysis.

Gas Chromatography – Mass Spectrometry (GC-MS) was conducted to detect the presence of formaldehyde in the samples. Separations of crude extracts were conducted using a 5% phenyl methylpolysiloxane (HP5-MS) fused capillary GC column with the measurements of 30 m × 0.32 mm in depth and × 0.25 µm film thickness with the Agilent GC system which comprises of Agilent 600 MS. Helium was used as carrier gas at a flow rate of 1.0 mL per min minute. The injection port and detector temperature were set at 280ºC and 300ºC, respectively.

Gas chromatography temperature profile was set as follows: initially temperature at 50ºC (held 1 min) ramped at 10ºC min^{-1} and increased to 280ºC (held 2 min).

Sample measuring 1 µL was injected using micropipette manually into the injection port under splitless mode with the solvent delay of 3.00 min. For the MS conditions, SCAN mode was selected with source temperature of 230°C, quad temperature at 150°C, transfer line temperature of 275°C and multiplier voltage auto tune voltage at 17 kV. SCAN mode is a computerized technique that detects mass spectral data found in a specific interval in relation with the voltage.

The acquired result of formaldehyde content in the sample was compared to the recommended formaldehyde level in fish which is about at most 5 ppm according to The Food Act 1983 and The Food Regulations 1985 of Malaysia. If there is a finding of formaldehyde level more than 5 ppm detected, it signifies that the fish is not safe for human consumption.

3.2.4 Statistical Analysis

All of the data collected were analysed by using Microsoft Excel.

4.0 RESULTS

4.1 Sensory Quality Assessment

The finding of the sensory quality assessment of Indian Mackerel was compared between the AEON Mall Supermarket (Supermarket) and Siti Khadijah Market (Wet market) based on Figure 4.1 and Figure 4.2 respectively. Two out of three Indian Mackerel from the wet market had firmer body to touch compared to the supermarket that had only one out of three Indian Mackerel which had a firm body to touch. Otherwise, only one out of three Indian Mackerel in wet market had a soft body to touch compared in supermarket, there were two out of three Indian Mackerel had a soft body to touch. Furthermore, all three Indian Mackerel from the wet market had a body that had quick recovery upon touch compared to in supermarket where only one out of three Indian Mackerel had a body with quick recovery upon touch. Moreover, there were none out of three Indian Mackerel in wet market had a body with slow recovery upon touch compared to in supermarket, there were two out of three Indian Mackerel had a body that had slow recovery upon touch. All three Indian Mackerel from both wet market and supermarket had body with iridescent shine to it. Apart from that, all three Indian Mackerel from both wet market and supermarket had a brownish gills and yellow mucus.

Besides that, none of the Indian Mackerel in wet market had reddened eye compared to one out of three Indian Mackerel from supermarket had reddened eyes. All the three Indian Mackerel from wet market had greyish and concaved eyes compared to the Indian Mackerel from supermarket where two out of three Indian Mackerel had greyish eyes while all three Indian Mackerel had concaved eyes. In addition, two out of three Indian Mackerel from wet market had firm abdomen compared to only one out of three Indian Mackerel in supermarket had a firm abdomen. Only one out of three Indian Mackerel from wet market had a soft abdomen compared to two out of three Indian Mackerel from supermarket had a soft abdomen.

Moreover, all three Indian Mackerel from wet market had a closed anus and none had ruptured anus compared to Indian Mackerel from supermarket where two out of three Indian Mackerel had ruptured anus while only one Indian Mackerel had a closed anus. Finally, all three Indian Mackerel from both wet market and supermarket had a fresh seaweed odour.

Furthermore, all three Indian Mackerel purchased from wet market has passed at least 8 out of 15 (53%) characteristics which is indicated in orange-coloured boxes in the bar graph. Meanwhile, all three Indian Mackerel purchased from supermarket only had passed 5 out of 15 (33%) quality characteristics which is indicated by the yellow-coloured boxes in the bar graph.

Figure 4.1 Bar graph of the sensory quality assessment obtained from Indian Mackerel collected from AEON Mall Supermarket (Supermarket) on three different days.

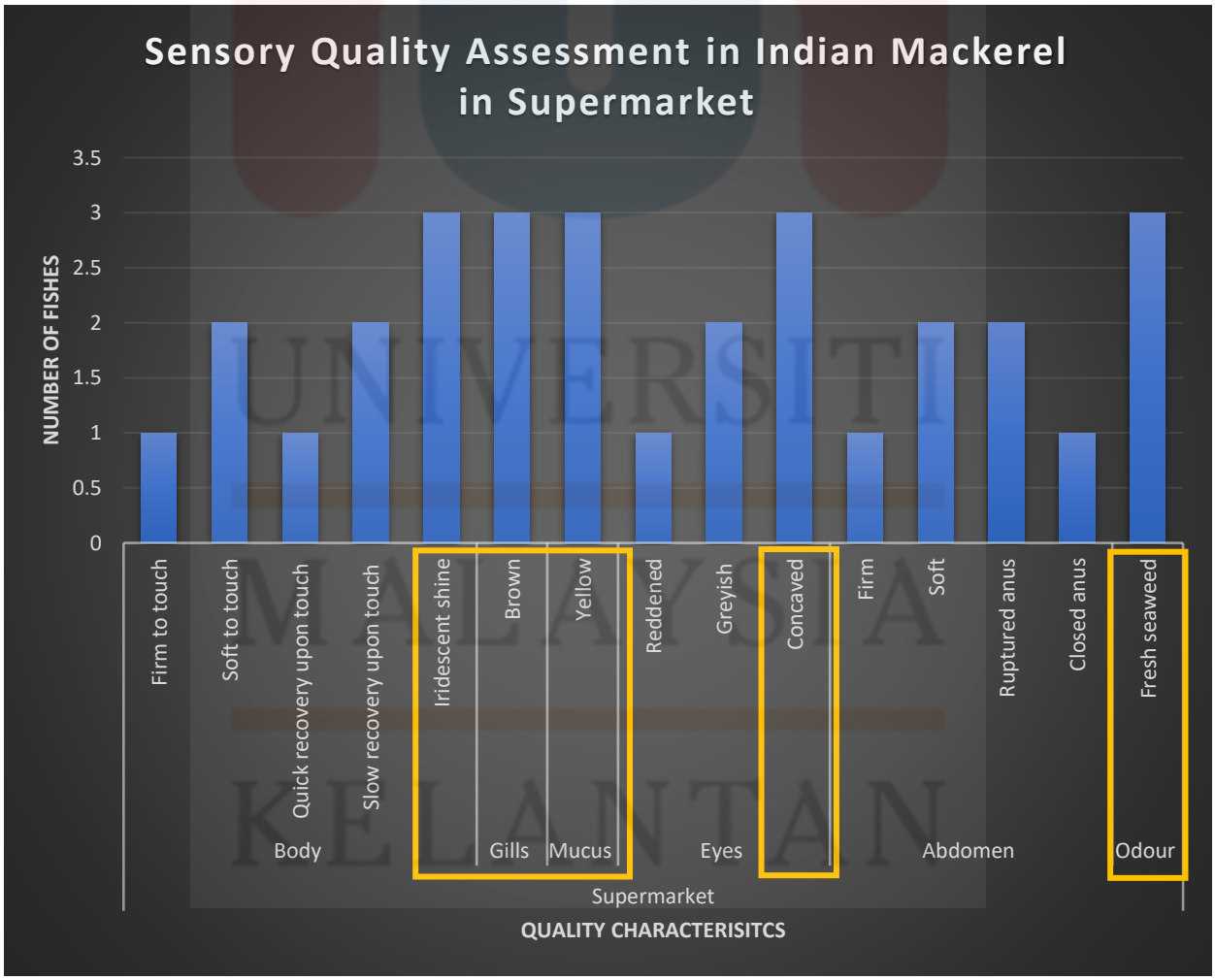
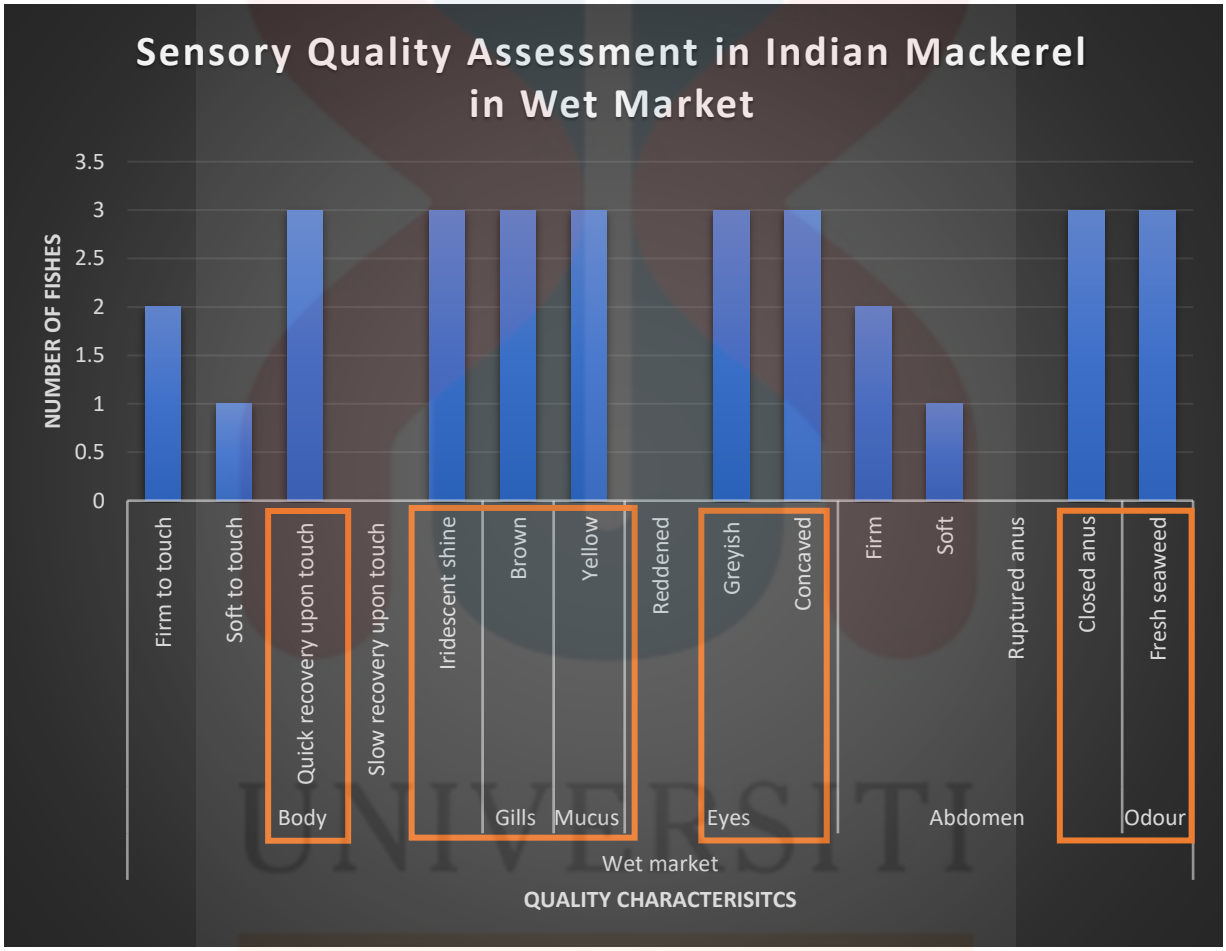


Figure 4.2 Bar graph of the sensory quality assessment obtained from Indian Mackerel collected from Siti Khadijah Market (Wet Market) on three different days.



4.2 Determination of pH

Table 4.1 below shows the pH obtained from the Indian Mackerel collected from Siti Khadijah Market and AEON Mall Supermarket. The pH 5 was consistent for all six samples collected on all three days. Therefore, the calculated mean value of the pH was pH 5.

Table 4.1 The pH value obtained from Indian Mackerel collected from AEON Mall supermarket and Siti Khadijah Market on three different days.

Species	pH values						Mean
	Markets			Supermarket			
	Siti Khadijah			AEON Mall			
Days	1st	2nd	3rd	1st	2nd	3rd	
Rastrelliger kanagurta (Indian Mackerel)	pH 5	pH 5	pH 5	pH 5	pH 5	pH 5	pH 5

4.3 Determination of Formaldehyde

Table 4.2 shows the result of qualitative analysis for presence of formaldehyde obtained from Indian Mackerel collected from both AEON Mall Supermarket and Siti Khadijah Market. The outcome of this detection was no presence of formaldehyde traces. The result was consistent with all six of the fish samples which were S1, S4, S5, M2, M3 and M5.

Table 4.2 The qualitative analysis for presence of formaldehyde obtained from Indian Mackerel collected from AEON Mall Supermarket and Siti Khadijah Market on three different days.

Species	Presence of Formaldehyde					
	Supermarket			Market		
	AEON Mall			Siti Khadijah		
Rastrelliger kanagurta (Indian Mackerel)	S 1	S 4	S 5	M 2	M 3	M 5
	None	None	None	None	None	None

Note: S1, S 4, S 5, M 2, M 3 and M 5 are labels for samples

Table 4.3 below shows the detection of other chemical compounds obtained from Indian Mackerel. The fish samples from AEON Mall Supermarket contained traces of Arsenous acid, tris(trimethylsilyl) ester and 7-Hexadecyn-1-ol in sample S1. Meanwhile, sample S5 contained traces of Heptasiloxane,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- and 1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-. However, sample S4 does not contain any traces of chemicals at all.

Based on the detection of other chemical compounds obtained from Indian Mackerel tabulated in Table 4.3 below, the fish samples from Siti Khadijah Market contained traces of Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- and Cyclobarbital in sample M3. Meanwhile, sample M5 contained traces of Heptasiloxane,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- only. However, sample M2 does not contain any traces of chemicals at all.

Table 4.3 The presence of other chemical compounds obtained from Indian Mackerel collected from AEON Mall Supermarket and Siti Khadijah Market on three different days.

Samples	Detected chemical compounds
AEON Mall Supermarket	
S1	1. Arsenous acid, tris(trimethylsilyl) ester 2. 7-Hexadecyn-1-ol
S4	None
S5	1. Heptasiloxane,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- 2. 1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-,
Siti Khadijah Market	
M2	None
M3	1. Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- 2. Cyclobarbital
M5	1. Heptasiloxane,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-

5.0 DISCUSSION

Formaldehyde is a naturally occurring chemical compound in the marine fish due to enzymatic reduction takes place after death. It is also evident that Trimethylamine oxide (TMAO) is responsible where it is enzymatically reduced into formaldehyde and dimethylamine. It is stated by Sotelo *et al.*, (1995), this compound accumulates within the fish cadaver where the cadaver reacts with protein resulting in protein denaturing and muscle toughness if it is not stored properly. From our investigation, we are unable to detect any presence of formaldehyde content in our all six fish samples from both the supermarket and market. This is an unusual finding due to the article references and previous studies suggest formaldehyde is a naturally occurring chemical compound in any fresh food such as fruits, vegetables and meat, especially marine fish.

Formaldehyde was not detected in this research and could be due to various factors. Primarily, there were alterations in the procedure that have been used to analyse formaldehyde content in this research. Although Gas Chromatography – Mass Spectrometry (GC-MS) used in this research is highly specific and sensitive compared to the predecessors, it always coupled with Solid Phase Microextraction (SPME) for the raw sample preparation to extract the formaldehyde from the fish tissue (Bianchi *et al.*, 2007). However, SPME was not carried out in this research instead, trichloroacetic acid was used for the extraction of the formaldehyde from the fish tissue**.** From the article references Noordiana *et al*., (2011) and Immaculate & Jamila (2016), this method has only been used along with the ultraviolet - visible spectroscopy coupled with Nash reagent to optimize the reaction to extract and detect formaldehyde from the fish sample. It is a commonly used method due to the reaction of Trichloroacetic acid (TCA) coupled with Nash reagent resulting in precipitation of the formaldehyde nano-molecules in ultraviolet spectrum (Kleeberg & Klinger, 1982).

Furthermore, chances of undesirable chemical reaction would have caused the absence of formaldehyde in the fish samples. For instance, Cannizaro reaction consumes the formaldehyde and reacts with potassium hydroxide (KOH) resulting in the formation of carboxylic acid and alcohol (Chemistry LibreTexts, 2016). In this project, a specific step in the formaldehyde extraction procedure requires stabilization and neutralisation of the final filtrate between pH 6 – pH 7 using Potassium hydroxide (KOH) and hydrochloric acid (HCl) with the aid of digital pH electrode meter. Though, the device malfunctioned due to technical errors resulting in the use of Universal pH Indicator Paper to supervise the continuous pH changes. Digital pH electrode meter is highly robust and sensitive to micro alterations of pH in the environment compared to the use of Universal pH Indicator paper which can be least sensitive and specific. Relying on the Universal pH Indicator paper could have led to excessive use of KOH and HCl resulting in unwanted chemical reaction altering the final filtrate leading to unexpected chemical compound formation.

Although there was no presence of formaldehyde in the sample, we were able to extract other chemical compounds from the GC-MS qualitative analysis. For instance, the traces of *Arsenous acid, tris(trimethylsilyl) ester*, *7-Hexadecyn-1-ol*, *Heptasiloxane,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-*, *1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-*, and *Cyclobarbital* were present. It is believed that these chemical compounds were formed due to extended chemical reactions between the muscle protein and formaldehyde with the presence of Trichloroacetic acid (TCA). In line with Metz *et al.*, (2004), formaldehyde is a well-known cross-linking agent that can inactivate, stabilize, or immobilize proteins by reacting with the amino group of the N-terminal amino acid residue and the sidechains of arginine, cysteine, histidine, and lysine residues forming methylene bridges. It is known that formaldehyde is a biological electrophile but the reactions between formaldehyde and biological components were not fully understood. However, there is a

significant finding suggests formaldehyde reacts at different rates, forming hydroxymethylated, cyclised, cross-linked, or disproportionated chemical products of varying stabilities (Kamps *et al.*, 2019).

The pH value plays an important role in determining the quality of the freshness in fish. As reported by Noordiana *et al.*, (2011), typical pH of live fish muscle is approximately 7.0. Based on the finding, all the 6 fish cadaver samples were detected for similar pH which was pH 5 indicating acidic environment. Slightly lower pH could be a result of the conversion of glycogen in the muscle to lactic acid whereas higher pH indicates storage period and spoilage of the fish (Kyrana & Lougovois, 2002). Apart from that, according to (Kayim & Can, 2010), the low muscle pH in the initial period reflects the fish is in a good nutritional state. To deduce, the fish acquired from both AEON Mall Supermarket and Siti Khadijah Market appears to be high in degree of freshness coincided with the pH value.

The sensory quality of the Indian Mackerel from the AEON Mall Supermarket, most of the fish had fresh seaweed odour, bilateral brownish gills, yellowish mucous and iridescent body shine indicating the fish has high degree of freshness. However, other organ assessments revealed concaved and reddened eyes, soft abdomen, and ruptured anus, soft to touch and slow recovery of the body indicated a slight drop in the fish freshness quality. Slightly hardened muscle texture resembles slow recovery of the body indicating breakdown of TMAO in muscle fibre has begun. The examination of other organs proves the fish sample from AEON Mall Supermarket is still in acceptable freshness range. To reiterate, fishes with less amount of formaldehyde cannot be detected through any objectionable sensory quality (Immaculate & Jamila, 2016). Furthermore, all three Indian mackerel purchased from the supermarket only passed 5 out of 15 (33%) quality characteristics. Thus, the fish from AEON Mall Supermarket was considered to be in good quality. The sensory quality assessment of the Indian Mackerel form Siti Khadijah Market reveals most of the fish had fresh seaweed odour, bilateral brownish

gills, concaved and greyish eye, yellowish mucous, firm abdomen and closed anus, firm to touch and quick recovery of the body and iridescent body shine. Thus, the fish from Siti Khadijah Market has higher degree of freshness and presented to be in excellent quality. The evident was all the three Indian Mackerel purchased from wet market has passed at least 8 out of 15 (53%) characteristics proving that the fish are in excellent freshness condition. With that it is safe to say, the Siti Khadijah Wet Market has higher degree of freshness quality compared to the AEON Mall Supermarket.

The presence of formaldehyde in fish higher than the recommended level which is 5 ppm is unsafe for human consumption. It is regulated by The Food Act 1983 and The Food Regulations 1985 of Malaysia. Surprisingly, this study recorded no formaldehyde in purchased Indian Mackerel in either wet market or supermarket indicating it is safe for human consumption.

6.0 CONCLUSION

In conclusion, there is no presence of formaldehyde traces in Indian Mackerel fish obtained from the markets. It has been proven that the Indian Mackerel obtained from the Siti Khadijah Wet Market is perceived to be in better quality compared to the AEON Mall Supermarket. Finally, the Indian Mackerel acquired from both market and supermarket are safe for human consumption.

7.0 RECOMMENDATION

There are several recommendations to improve this study in the future. It is recommended to increase the sample size from six to at least thirty samples to have a better conclusive outcome. More samples directly proportional to higher specificity and higher reliability. Apart from that, the rooms for errors must be minimized close to zero especially when handling GC-MS machine due to its high specificity and sensitivity. Finally, proper instruments must be used to achieve an accurate result. For instance, using a digitalised pH electrode meters significantly increases the accuracy of the pH for reliability and also reduces time consumption instead of using a Universal Indicator Paper pH.

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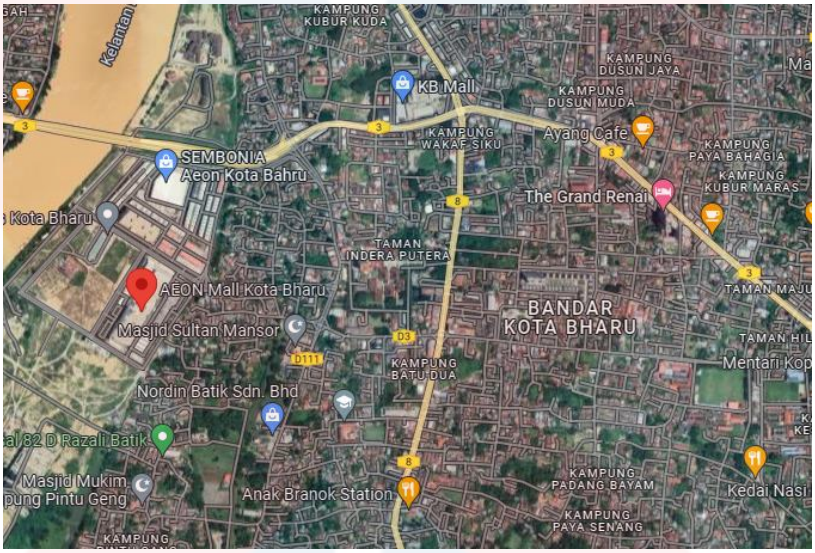
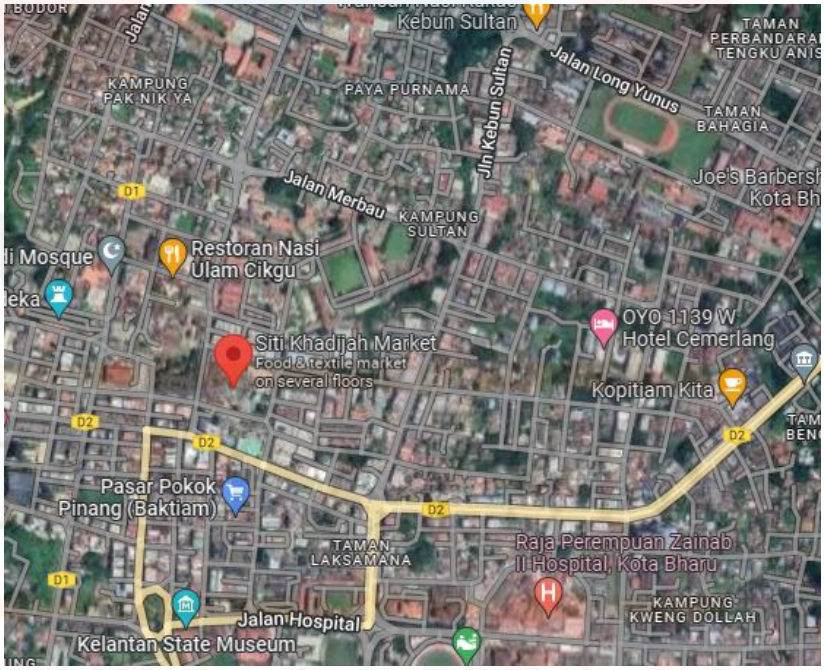
Appendix A

Appendix A.1: The satellite view of AEON Mall Kota Bharu



Appendix A.2: The satellite view of Siti Khadijah Market Kota Bharu



Appendix A.3: The body length and body weight obtained from AEON Mall Supermarket.

AEON Mall Supermarket			
No	Identity of Fish	Body length (cm)	Body weight (g)
1	Fish 1	28.1	294
2	Fish 2	26.8	254
3	Fish 3	27.3	258

Appendix A.4: The body length and body weight obtained from Siti Khadijah Market

Siti Khadijah Market			
No	Identity of Fish	Body length (cm)	Body weight (g)
1	Fish 1	28.0	312
2	Fish 2	24.5	176
3	Fish 3	22.3	138

Appendix A.5: The fish measuring method using a ruler and a cutting board.

Appendix A.6: The Universal Indicator Paper used for pH detection.